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Disruption and aberrant expression of *HMGA2* as a consequence of diverse chromosomal translocations in myeloid malignancies

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Chromosomal translocations that target *HMGA2* at chromosome band 12q14 are seen in a variety of malignancies, notably lipoma, pleomorphic salivary adenoma and uterine leiomyoma. Although some *HMGA2* fusion genes have been reported, several lines of evidence suggest that the critical pathogenic event is the expression of truncated HMGA2 isoforms. We report here the involvement of *HMGA2* in six patients with myeloid neoplasia, dysplastic features and translocations or an inversion involving chromosome bands 12q13–15 and either 7p12, 8q22, 11q23, 12p11, 14q31 or 20q11. Breaks within or very close to *HMGA2* were found in all six cases by molecular cytogenetic analysis, leading to overexpression of this gene as assessed by RT-PCR. Truncated transcripts consisting of *HMGA2* exons 1–2 or exons 1–3 spliced to intron-derived sequences were identified in two patients, but were not seen in controls. These findings suggest that abnormalities of *HMGA2* play an important and previously unsuspected role in myelodysplasia.
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Introduction

The high-mobility group (HMG) proteins HMGA1a, HMGA1b and HMGA2 comprise a subgroup of nonhistone chromatin accessory proteins, often referred to as architectural transcription factors. [1–3] They are low molecular mass nuclear proteins of about 100 amino acids, which each possess three copies of a nine amino-acid motif (AT-hook) that interacts with the minor groove of many promoter and enhancer DNA regulatory elements.[4] HMG proteins possess no intrinsic transcriptional activity, but instead function to orchestrate the assembly of nucleoprotein structures involved in gene replication, transcription and overall chromatin structure through a complex network of protein–DNA and protein–protein interactions.[1–7]

The *HMGA2* gene (formerly known as *HMGI-C*) spans a genomic region of 160 kb at chromosome band 12q13 and consists of five exons. The translated protein contains the three AT hooks, encoded by exons 1–3, and an acidic C-terminal domain, encoded by exon 5.[8] *HMGA2* is expressed predominantly during embryonic development,[8–14] but is overexpressed in many malignant cell lines, and is thought to contribute to the transformation process.[9–11] The role of *HMGA2* in mouse development is underscored by the finding that inactivation of this gene results in the *pygmy* mouse, the phenotype of which exhibits growth retardation and a significant reduction of overall body adipose tissue.[12]

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Rearrangements of *HMGA2* have been detected frequently in human mesenchymal tumors, resulting in fusion to diverse partner genes. In lipomas, *HMGA2* has been shown to fuse to *LPP* at 3q27–q28, a gene encoding an LIM domain-containing protein;[8,15] to *LHFP* at 13q12, the function of which is still unknown;[16] to the G-protein-coupled receptor *RDC1* at 2q35–37[17] and to a putative gene at 15q24 predicted to encode a protein with a serine/threonine-rich domain.[18] In pleomorphic adenomas of the parotid gland, *HMGA2* is fused to *FHIT* at 3p14, the gene that is frequently disrupted in gastrointestinal tumors[19,20] and to *NFIB* at 9p24.1, a member of the human nuclear factor I gene family.[21] In osteosarcoma, *HMGA2* is fused to the proteoglycan *LUM* gene at 12q22–23,[22] whereas in uterine leiomyomas fusion partners include the mitochondrial aldehyde dehydrogenase gene *ALDH2* at 12q24.1,[23] the recombinational repair gene *RAD51L1* at 14q23–24[24] and the cytochrome *c* oxidase subunit *COX6C* at 8q22–23.[25]

Of these fusions, only *HMGA2-RAD51L1* and *HMGA2-LPP* yield in frame chimeric transcripts. All other fusions are out of frame and predicted to be translated into truncated variants of HMGA2. The breakpoints in the gene are typically located in introns 3 or 4, with the translated truncated products predicted to retain the AT hooks but to have lost the C-terminal acidic domain. In addition to direct truncation by gene fusion, several chromosomal translocations involving *HMGA2* have been shown to result in the expression of aberrantly spliced transcripts that may also encode truncated forms of HMGA2. These mRNAs are structurally similar to those formed by gene fusion, typically consisting of *HMGA2* exons 1–3 fused to intronic sequences from the same gene.[8,26] These mRNA variants have been found in all the tumor types described above.[8,26–29]

These findings, and the fact that there is no obvious functional relationship between the various partner genes, suggest that overexpression of the N-terminal part of HMGA2 may be the critical transforming event in tumors with 12q13 rearrangements, rather than the formation of particular fusion genes. This idea is supported by functional analysis using both cell lines and animal models.[30–32] However, overexpression of wild-type *HMGA2* also predisposes to malignancy[33] and it has been suggested that truncated HMGA2 proteins are transforming by virtue of the fact that they activate expression of the wild-type *HMGA2* allele.[34]

Chromosomal translocations are common in hematological malignancies and typically generate transforming oncogenes by gene fusion or overexpression of one or more genes near the breakpoints. Despite the diversity of chromosomal translocations in patients with leukemia or lymphoma, direct involvement of *HMGA2* has only been reported in four cases, one with Richter transformation of chronic lymphocytic leukemia (CLL),[35] one with acute lymphoblastic leukemia (ALL)[27] and two with myelofibrosis with myeloid metaplasia (MM).[28] Here, we report six cases of myeloid malignancy with reciprocal translocations involving 12q13–15 and involvement of *HMGA2*.

Patients and methods

Patient ascertainment

In the 10-year period from 1994 to 2003, the hematological malignancy cytogenetics services in Pamplona and Salisbury analyzed 13 582 new cases, of which 4396 (32%) were abnormal. Of these abnormal karyotypes, we noticed a recurrent site of breakage at 12q13–q15 in 0.8% of all cases, corresponding to 2.2% of those that had an abnormal karyotype. Given the known involvement of *HMGA2* in mesenchymal tumors, we hypothesized that this gene may also be targeted in hematological malignancies. Patients with 12q13–15 abnormalities were selected for further analysis on the basis of availability of stored pretreatment fixed cells. Brief clinical details on the six cases for whom *HMGA2* rearrangements were identified are given below.

Case 1: A 33-year-old female with myelodysplastic syndrome (MDS), subtype refractory anemia with excess of blasts (RAEB-1). Cytogenetic analysis of bone marrow (BM)-derived metaphases showed 46,XX[18]/46,XX,t(7;12)(p12;q13)[12] and although the proportion of malignant cells in her BM karyotype increased to 100%, the patient remains largely asymptomatic 5 years after presentation.

Case 2: A 63-year-old male with MDS, subtype RAEB-2 who presented with peripheral blood counts of hemoglobin 10 g/dl, WBC 13×10^9/l (11% blasts), platelets 39×10^9/l. BM karyotype showed 46,XY[24]/46,XY,t(12;14)(q13;q31)[6]. The patient died 6 months after presentation due to respiratory and other complications.

Case 3: A 72-year-old female was admitted to hospital due to progression of normocytic, normochromic anemia to MDS, subtype RAEB-1. Peripheral blood counts were: hemoglobin 9 g/dl, WBC 6×10^9/l (15% blasts), platelets 320×10^9/l with 3% blasts. Cytogenetic analysis showed 46,XX,t(12;12)(p13;q13) in all 30 metaphases and the possibility of a constitutional translocation was eliminated by karyotyping a PHA-stimulated peripheral blood sample. The patient died 3 months after presentation after having progressed to acute myeloid leukemia (AML).

Case 4: A 67-year-old female with myelodysplastic/myeloproliferative disease, unclassifiable (MDS/MPD-U). BCR-ABL was excluded by RT-PCR analysis. Peripheral blood counts at presentation were: hemoglobin 85 g/dl, WBC 45×10^9/l (15% blasts), platelets 469×10^9/l. Notable features included massive splenomegaly, pronounced basophilia (10% basophils in the peripheral blood) and BM fibrosis. Cytogenetic analysis showed 46,XX,add(6)(p2?2),t(12;20)(q15;q11.2) in all 30 BM metaphases and analysis of PHA-stimulated cultures revealed these abnormalities to be acquired. At 20 months after presentation, the patient remains largely asymptomatic apart from occasional requirement for blood transfusion due to low hemoglobin levels.

Case 5: A 53-year-old female with MDS/MPD, subtype atypical chronic myeloid leukemia (aCML). BCR-ABL was excluded by RT-PCR analysis. Peripheral blood counts at presentation were: hemoglobin 12 g/l, WBC 31×10^9/l, platelets 267×10^9/l. Massive splenomegaly and gum hypertrophy were noted and cytogenetic analysis showed 47,XXX,t(8;12)(q22;q13) in all 10 BM metaphases analysis, three of which also had del(13)(q12q14). As above, analysis of PHA-stimulated cultures showed that the abnormalities were acquired.

Case 6: A 48-year-old female presented with AML-M0 and a normal karyotype. BCR-ABL was excluded by RT-PCR analysis. Remission was achieved following chemotherapy, but she relapsed 4 years later with MDS, subtype RAEB-1. Cytogenetic analysis of BM metaphases showed 46,XX,t(11;12)(q23;q15)[7]/46,XX[6]. After two BM autografts, the patient died 6 months later and after having progressed to AML-M2.

These cases are summarized in Table 1.

Table 1 Summary of cases analyzed

Patient	*Translocation*	*Diagnosis*
Case 1	t(7;12) (p12;q13)	MDS (RAEB1)
Case 2	t(12;14) (q13;q31)	MDS (RAEB2)
Case 3	t(12;12) (p11;q13)	MDS (RAEB1)
Case 4	t(12;20) (q15;q11.2)	MDS/MPD (U)
Case 5	t(8;12) (q22;q13)	MDS/MPD (aCML)
Case 6	t(11;12) (q23;q15)	Secondary MDS (RAEB1)

Fluorescence in situ *hybridization (FISH)*

YAC, BAC and PAC clones were identified from database searches and obtained from the BACPAC Resource Center at the Children's Hospital Oakland Research Institute (Oakland, CA, USA), the Sanger Institute (Hinxton, UK) or the Human Genome Mapping Project (Hinxton, UK). Clones were grown, labelled and hybridized to patient metaphases using standard procedures. To localize the breakpoint more precisely for case 4, a probe was generated by long PCR from BAC RP11-366L20 DNA using the High Fidelity PCR Master kit (Roche, Mannheim, Germany) and primers HMGexon41F and HMGexon51R. The amplification reaction included biotin-16-dUTP at a final concentration of 10 μM, and the 12 kb product was partially digested with DNAse 1 using standard procedures prior to hybridization to patient metaphases.

Nucleid acid isolation

Total RNA was extracted from fixed cells using the Total RNA Isolation Kit (Qiagen, West Sussex, UK). cDNA was generated with the SuperScript II RNase H-Reverse Transcriptase (RT) (Invitrogen Life Technologies Inc., Gaithersburg, MD, USA) and was used as a template for PCR. The quality of cDNA in the patient sample was confirmed by amplification of the *G3PDH* or *BCR* genes.

Rapid amplification of cDNA ends (RACE) PCR

3′ RACE PCR was performed on patient and normal control RNA using the Gene Racer kit (Invitrogen BV, Groningen, The Netherlands). Briefly, first strand cDNA was reverse-transcribed from total RNA using SuperScript II RNase H-RT (Invitrogen Life Technologies, Inc.) and the GeneRacer Oligo dT Primer. This cDNA was then amplified using *HMGA2* gene-specific forward primers (HM1643F, HM2366F, HA2EX1F or HMGexon21F) and the GeneRacer 3′ Primer. For some cases, nested PCR was performed using the GeneRacer 3′ Nested Primer as the reverse

Table 2 Oligonucleotide primer sequences

Name	*Gene*	*Sequence*
HA2EX1F	*HMGA2* exon 1	5′-CGCCTAACATTTCAAGGGACACA-3′
HA2EX1RN	*HMGA2* exon 1	5′-CACTCCAAGTCTCTTCCCTTTCCAA-3′
HMGexon14F	*HMGA2* exon 1	5′-CAGCGCCTCAGAAGAGAGGACG-3′
HMRADFOR	*HMGA2* exon 2	5′-GGAAGACCCAAAGGCAGCAA-3′
HMRADFORN	*HMGA2* exon 2	5′-AAGGCAGCAAAAACAAGAGT-3′
HMGexon21F	*HMGA2* exon 2	5′-AACCAACCGGTGAGCCCTCTCCT-3′
HC1R	*HMGA2* intron 2	5′-GTCAAGAAACTCCAAGCAGCAAG-3′
HA2EX3R	*HMGA2* exon 3	5′-TTTTTCTCCAGTGGCTTCTGCT-3′
HMGexon41F	*HMGA2* exon 4	5′-GTTGTTCAGAAGAAGCCTGCTCA-3′
HM1643F	*HMGA2* exon 5	5′-CACCTCAAATACCACCCCAACC-3′
HM2366F	*HMGA2* exon 5	5′-TTCGGCTTGAGTTCACCATCTCT-3′
HM3759F	*HMGA2* exon 5	5′-GGCCAATGGAACAGTAAGAACATC-3′
RAHMREV	*HMGA2* exon 5	5′-CACCCCACCCCAGATGAAAG-3′
RAHMREVN	*HMGA2* exon 5	5′-CTTCGGCAGACTCTTGTGAGG-3′
HMGexon51R	*HMGA2* exon 5	5′-CTCTTGTGAGGATGTCTCTTCAG-3′
RAHMFOR	*RAD51L1* exon 5	5′-ATAATGATGAGCATTTTGGCTACA-3′
RAHMFORN	*RAD51L1* exon 5	5′-TGATGAGCATTTTGGCTACATTA-3′
HMRADREV	*RAD51L1* exon 10	5′-CTCCTTGATGGTGTAGACAAATGAG-3′
HMRADREVN	*RAD51L1* exon 10	5′-TGGTGTAGACAAATGAGGTGAA-3′

primer, and HM3759F or HA2EX1RN as the forward primers. In both reactions, after initial denaturation at 94°C for 10 min, 35 cycles at 94°C for 1 min, at 65°C for 1 min and at 72°C for 1 min were used, followed by a final elongation at 72°C for 10 min. The primers were carefully designed to be compatible with the primer included in the kit. The reactions were supplemented with 0.3 mM of betaine in order to facilitate the amplification due to the high percent of GC in the *HMGA2* mRNA. The sequences of the primers are shown in Table 2.

RT-PCR

Total RNA from the BM cells of the patients and from BM, PBLs and lung from a healthy donor were used for cDNA synthesis using SuperScript II RNase H-RT (Invitrogen Life Technologies, Inc.) with random hexamer primers. PCRs were carried out with AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA) after optimizing cycling conditions for each primer pair. Expression of *HMGA2* was analyzed using primers HA2EX1F (exon 1) and HA2EX3R (exon 3) or HMGA2exon14F (exon 1) and HMGA2exon51R (exon 5). To look for the *HMGA2-RAD51L1* fusion product in case 2, RT-PCR was performed with primers HMRADFOR and HMRADREV. A nested PCR of this product was performed using primers HMRADFORN and HMRADREVN primers. Similarly, RT-PCR was performed to amplify the reciprocal *RAD51L1-HMGA2* fusion transcript using primers RAHMFOR and RAHMREV for the first PCR and RAHMFORN and RAHMREVN for the nested PCR. For case 4, the presence of a truncated *HMGA2* transcript was confirmed by RT-PCR with primers HMGexon21F and HC1R. All PCRs were performed for 30–35 cycles.

DNA cloning and sequencing

PCR products were cloned into the pCR4-TOPO vector (TOPO TA Cloning Kit for Sequencing; Invitrogen) following the manufacturer's instructions. Clones were sequenced using either BigDye Terminator or d-Rhodamine Terminator Cycle Sequencing Kits (Applied Biosystems, Foster City, USA) and fractionated on an ABI 377 or 3100 genetic analyzer (Applied Biosystems, Foster City, USA).

Results

Rearrangements of HMGA2

Of the patients identified with 12q13–15 rearrangements, fixed cells were available for analysis in 13 cases. Of these, eight did not show *HMGA2* involvement (CLL, $n=2$; ALL, $n=1$; CML-BC, $n=3$; MDS, $n=1$; CMPD, $n=1$) and these cases are not considered further here. FISH analysis of five patients, however, did indicate involvement of *HMGA2*. A sixth case ascertained from an independent series was also found to have an *HMGA2* rearrangement.

Cases 1–3 were analyzed with BAC RP11-462A13, which covers the entire *HMGA2* coding sequence, and BAC RP11-427K2, which contains *HMGA2* exons 4, 5 and downstream sequences (Figure 2). As expected, both clones hybridized to band q14 on normal copies of chromosome 12 (Figure 1). In addition, for case 1, RP11-462A13 hybridized to both derivative chromosomes, whereas RP11-427K2 hybridized to the der(7) only, suggesting a break upstream of *HMGA2* exon 4. For case 2, RP11-462A13 hybridized to both derivative chromosomes, indicating a break within *HMGA2*. For case 3, RP11-462A13 hybridized to one der(12), whereas RP11-427K2 hybridized to the other der(12), suggesting a break within *HMGA2* within or close to the small region of overlap between the two clones (Figure 1).

Cases 4–6 were analyzed with various probes in combination with BAC RP11-366L20, which covers approximately 99 kb of *HMGA2* intron 3, within which most genomic breakpoints that disrupt this gene have been reported to lie, plus exons 4 and 5 (Figure 2). For case 4, BAC RP11-366L20 hybridized to both derivative chromosomes and BAC RP11-745O10 was translocated to the der(20) (not shown). A PCR-generated probe spanning *HMGA2* exons 4 and 5 remained on the der(12), whereas RP11-118B13 was translocated to the der(20) (Figure 1), suggesting a break within the 3′UTR or shortly downstream of *HMGA2*. For cases 5 and 6, BAC RP11-366L20 hybridized to

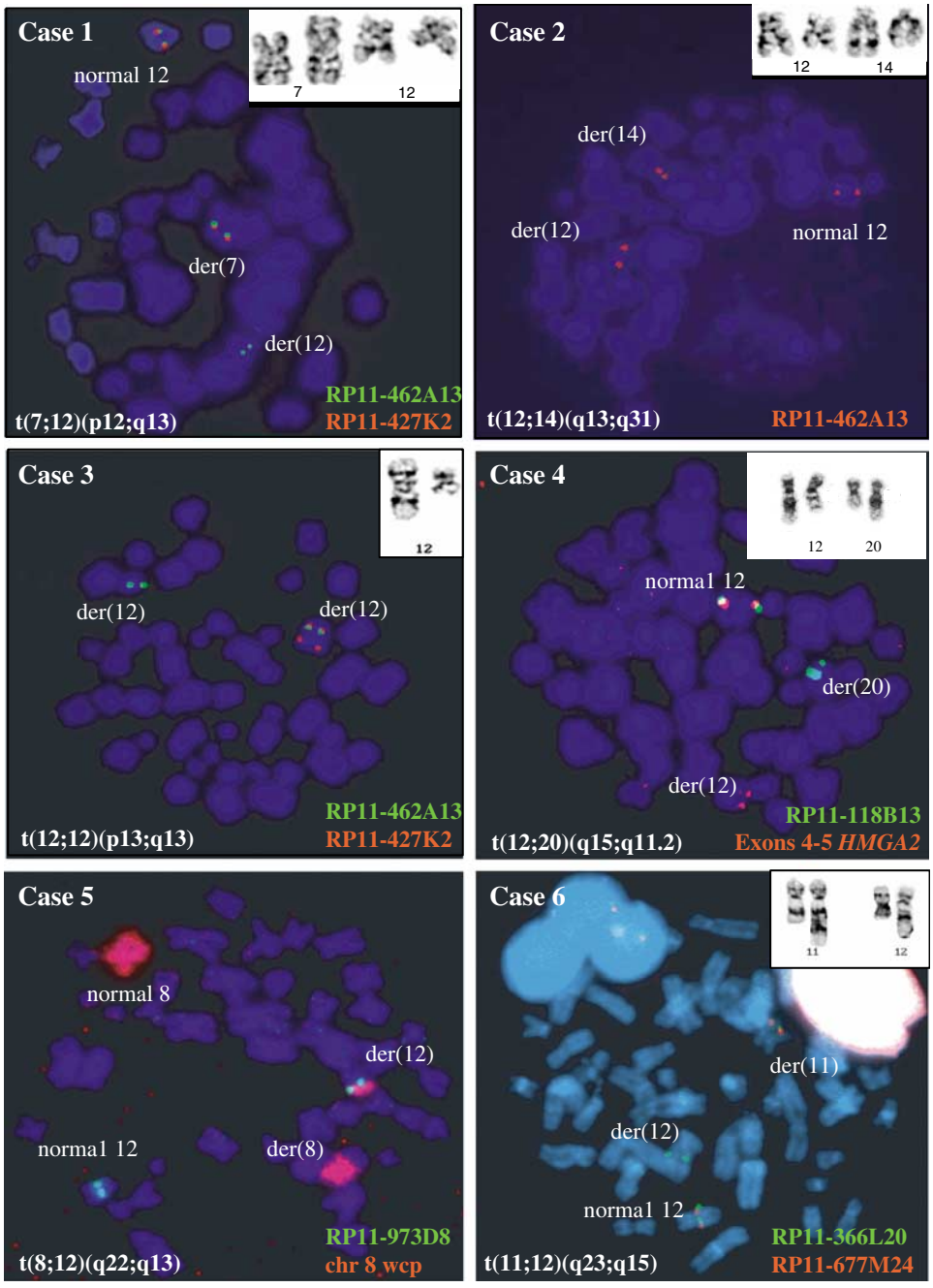

Figure 1 G-banding and FISH analysis for the six patients showing *HMGA2* rearrangement in cases 1–4 and 6 and illustrating the translocation for case 5. The probes and color used for their detection is indicated.

both derivative chromosomes (not shown and Figure 1), indicating a break within *HMGA2* downstream of exon 3.

Positions of breakpoints on partner chromosomes

FISH analysis also enabled us partially to define the breakpoints in the other chromosomes involved in the translocations, although lack of patient material precluded detailed analysis. For case 1, the breakpoint on 7p12 was located between RP11-109E9 and RP11-673M15, a distance of approximately 11 Mb that includes the *CDC10*, *RALA* and *GLI3* genes. For case 2, the breakpoint on 14q31 was between RP11-63G22 and RP11-140I4, a 27 Mb interval that contains *RAD51L1*, a known *HMGA2* partner gene.[24] For case 3, the breakpoint on 12p13 was mapped between RP11-117D20 and RP11-36O2, a distance of 3.2 Mb that includes *CCND2*. For case 5, the breakpoint was mapped to YAC 804c6 in the vicinity of D8S539, a region that contains a single very large gene called *CSMD3*, the function of which is currently unknown.

RT-PCR analysis

To test if *HMGA2* was expressed in patients with chromosomal translocations that target this gene, we performed RT-PCR

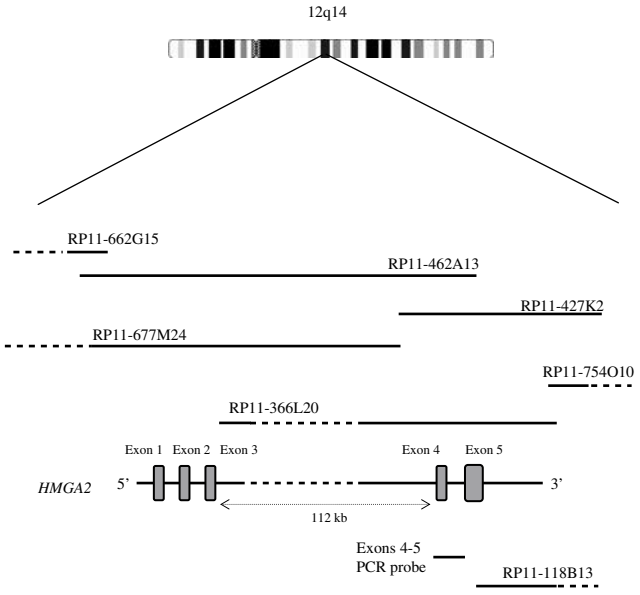

Figure 2 Map of 12q FISH probes used to define the breakpoint around and within *HMGA2* (not to scale).

analysis using primers directed against exons 1 and 3. *HMGA2* mRNA was readily detectable in normal lung tissue, used as a positive control, in BM from cases 1 to 3 (Figure 3a) and in BM from cases 4 to 6 (not shown). As expected, no expression of *HMGA2* was found in normal BM or peripheral blood. Cases 1–3 were also tested and found to be positive for *HMGA2* expression with primers to exons 1 and 5 (not shown).

RACE-PCR

To identify potential *HMGA2* fusion partners, we performed 3′ RACE PCR on cases for whom adequate RNA was available for analysis. For case 1, a PCR product of 392 bp was obtained (Figure 3b), the sequence of which showed *HMGA2* exon 3 spliced to intron 3-derived sequence from the same gene. If translated, this transcript would be predicted to encode a truncated *HMGA2* protein with normal amino acids encoded by exons 1–3, followed by an additional 12 amino acids encoded by the intron sequence (Figure 4). For cases 2 and 3, we failed to identify any novel fusions, but the material available for analysis in these individuals was very limited. Since the breakpoint in case 2 had been mapped to an interval that contained a known *HMGA2* partner, *RAD51L1*, we performed RT-PCR to look for *HMGA2-RAD51L1* and reciprocal *RAD51L1-HMGA2* fusion transcripts, however the results were negative.

For case 4, two RACE products were obtained (Figure 3c). The larger and most prominent band (1008 bp) again contained *HMGA2* exon 3 spliced to intron 3-derived sequence. This transcript is predicted to encode a truncated protein consisting of normal HMGA2 amino acids encoded by exons 1–3, followed by an additional seven amino acids encoded by the intron sequence (Figure 4). This truncated product is identical to that previously described in a human leiomyoma cell line with a t(12;14)(q15;q24).[8] The second, smaller 819 bp PCR product contained exon 2 of *HMGA2* spliced to 802 bp of intron 2. This sequence could also encode a truncated product with 15 amino acids added to a polypeptide encoded by the first two exons of *HMGA2* (Figure 4). Single step RT-PCR confirmed that the fusion

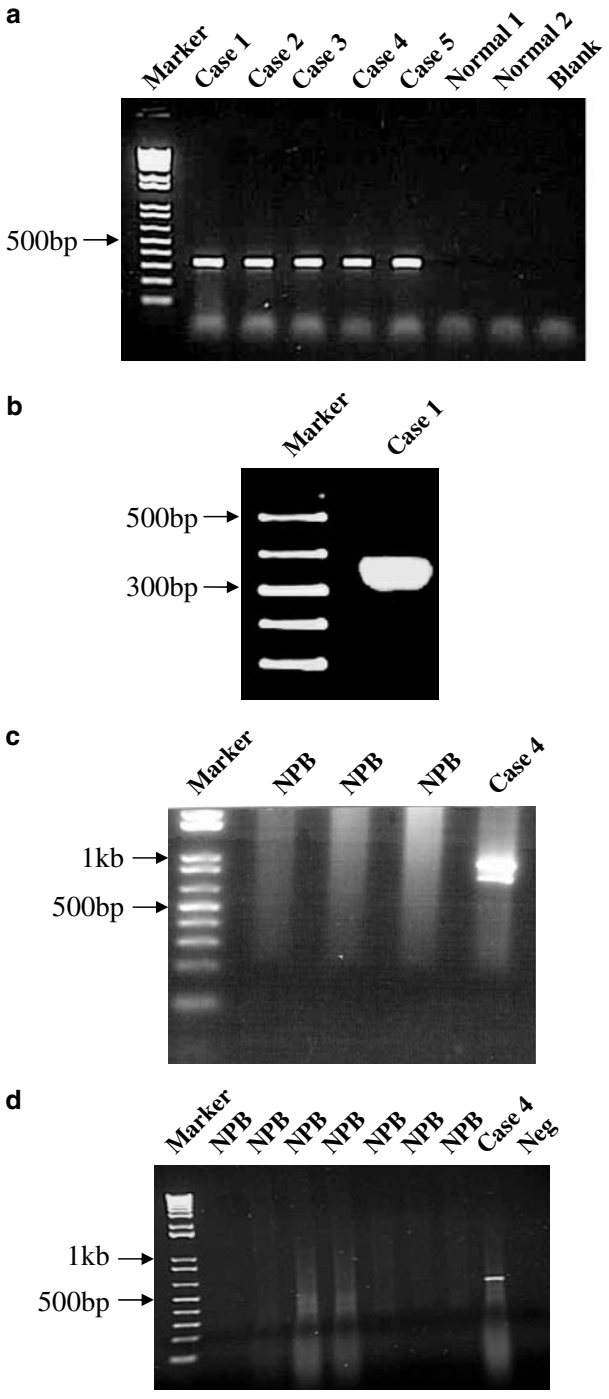

Figure 3 *HMGA2* RT-PCR and RACE analysis. (a) Expression of *HMGA2* in cases 1–5 (case 6 also expressed *HMGA2* but is not shown), but not normal controls using primers to exons 1 and 3. (b) RACE PCR for case 1. (c) RACE PCR for case 4. (d) RT-PCR showing specific amplification of the smaller truncated *HMGA2* mRNA in case 4. Abbreviations are: neg, negative control; NBM, normal bone marrow; NPB, normal peripheral blood.

products were not expressed in the blood of normal individuals, but were expressed in case 4 (Figure 3d and not shown). As described above, FISH analysis of this case indicated a breakpoint downstream of *HMGA2* coding sequence. Similar aberrant splicing has been described in uterine leiomyomata cases with breaks downstream of HMGA2,[29] suggesting that the

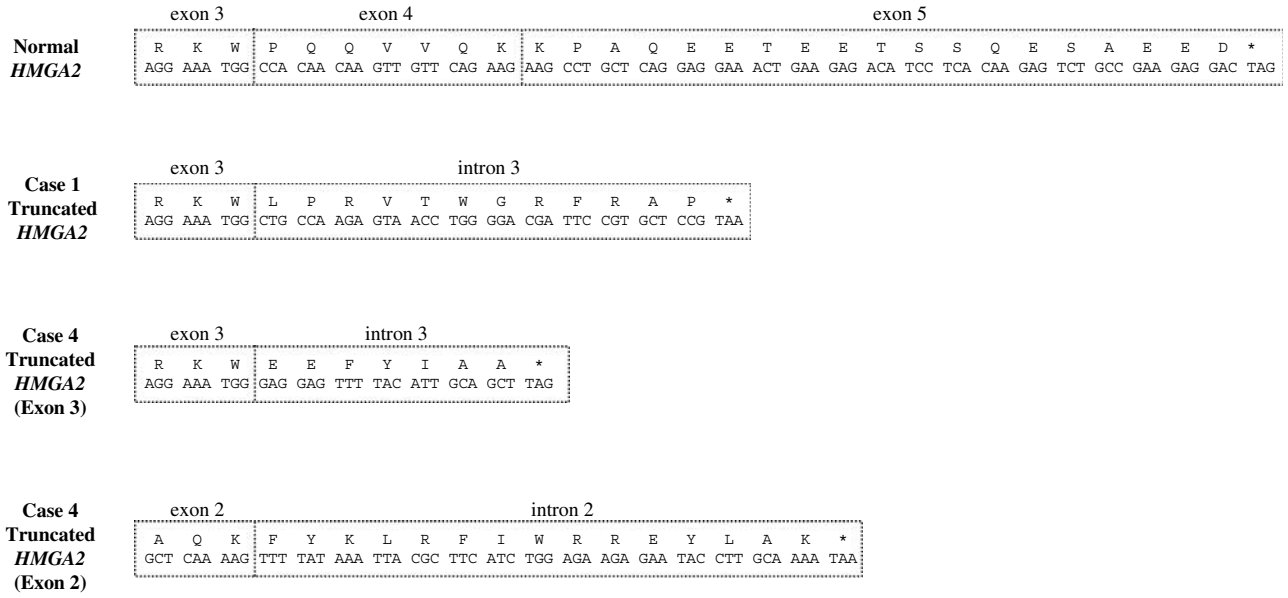

Figure 4 Structure of the normal *HMGA2* mRNA, and the truncated mRNAs found in cases 1 and 4.

splicing control of this gene is complex and may be disrupted by translocation into a novel locus. Alternatively, a small deletion accompanying the translocation that is not detected by our FISH probes could be present, resulting in the observed aberrant transcripts.

Discussion

We report here rearrangement and overexpression of the *HMGA2* gene in six patients with myeloid leukemia and a translocation involving 12q13–15. Strikingly, all six had dysplastic features and, to our knowledge, this is the first report of rearrangement and ectopic overexpression of *HMGA2* gene in patients with MDS. Although we cannot accurately determine the incidence of *HMGA2* involvement in MDS from our data, we can make a crude estimate. Analysis of 2891 MDS patients referred to the Pamplona and Salisbury diagnostic services for cytogenetic analysis indicates that 11 (0.4% of all cases; 1.5% of those with an abnormal karyotype) had rearrangements of 12q13–15 (unpublished data). Although the numbers analyzed in this study were small, we found that most patients with MDS and 12q13–15 rearrangements involved *HMGA2*. Thus, we anticipate the incidence of cytogenetically visible abnormalities of *HMGA2* to be of the order of 0.3–0.4% of all MDS cases.

FISH analysis indicated breaks within the *HMGA2* coding sequence for five cases, whereas in one case the breakpoint was in the 3′UTR, a region containing regulatory elements that exert post-transcriptional expression control.[36] Ectopic expression of *HMGA2* was found in all six patients, and expression of truncated isoforms was found in two of four cases analyzed. For case 1, *HMGA2* exon 3 was spliced to an intron 3-derived sequence that has not previously been described in any aberrant transcripts from this gene. Two truncated transcripts were found for case 4, one with *HMGA2* exon 3 spliced to intron 3 sequence, and the second with *HMGA2* exon 2 spliced to intron 2-derived sequence. The intron 3 sequence in case 4 is different from that seen in case 1 but is the same as that spliced to *HMGA2* in a human leiomyoma cell line with a t(12;14)(q15;q24).[8] The smaller transcript (*HMGA2* exon 2 spliced to intron 2) is novel and is predicted to be translated into a protein with just two of the three normal AT hooks. A structurally similar protein ($HMGA2_{73}$) was able to enhance the binding of the p50/p65 NF-κB heterodimer to the PRDII element of the IFN-β promoter,[37] suggesting that both the truncated products seen in case 4 might have biological activity.

HMGA2 expression in adult tissues is normally restricted to lung, kidney and synovia,[38–40] and reactivation of this gene has been previously associated with the development of benign tumors of mesenchymal origin as well as in sporadic cases of leukemia. Recently, quantitative real-time PCR analysis has shown that *HMGA2* transcripts are overexpressed in CD34+ cells from patients with myelofibrosis with myeloid metaplasia in comparison with normal CD34+ cells from healthy donors.[28] This dysregulation could be interpreted as a consequence of the malignant proliferative alteration of these cells and might well be an important step in the pathogenesis of this disorder. Our findings suggest that *HMGA2* may be more widely involved in hematological malignancies.

The molecular mechanism by which these chromosomal abnormalities and the aberrant expression of *HMGA2* lead to cancer still remains unknown, although recent data has implicated HMGA2 in the transcriptional regulation of cell cycle and DNA repair genes.[41,42] In cases with an in-frame chimeric fusion gene, it appears that only the translocated allele is expressed. However, overexpression of wild-type *HMGA2* mRNA has been found in uterine leiomyomas[29] as a consequence of rearrangements located upstream of this gene and furthermore it has been suggested that expression of the wild-type allele is critical to the pathogenesis of mesenchymal tumours with rearrangements of *HMGA2* that generate truncated variants.[34] Consistent with this idea, we observed expression of full-length *HMGA2* mRNA (exons 1–5) in three of three patients tested including case 1, in whom the translocation breakpoint had been mapped unambiguously to *HMGA2* intron 3.

In conclusion, this study demonstrates *HMGA2* rearrangement and overexpression in MDS patients with chromosomal

translocations involving 12q13–15. Our findings underscore the importance of this gene in malignancy and highlight the need for further investigations in order to understand the complex biology underlying the genesis, growth and development of myeloid neoplasias.

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